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journal homepage: www.elsevier.com/locate/envpolLong-term persistence of various ^{14}C -labeled pesticides in soilsNicolai D. Jablonowski^{b,*}, Andreas Linden^a, Stephan Köppchen^a, Björn Thiele^{b,c}, Diana Hofmann^a, Werner Mittelstaedt^a, Thomas Pütz^a, Peter Burauel^a^aInstitute of Bio- and Geosciences, IBG-3 – Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany^bInstitute of Bio- and Geosciences, IBG-2 – Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany^cBioSpec, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

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ABSTRACT

The fate of the ^{14}C -labeled herbicides ethidimuron (ETD), methabenzthiazuron (MBT), and the fungicide anilazine (ANI) in soils was evaluated after long-term aging (9–17 years) in field based lysimeters subject to crop rotation. Analysis of residual ^{14}C activity in the soils revealed 19% (ETD soil; 0–10 cm depth), 35% (MBT soil; 0–30), and 43% (ANI soil; 0–30) of the total initially applied. Accelerated solvent extraction yielded 90% (ETD soil), 26% (MBT soil), and 41% (ANI soil) of residual pesticide ^{14}C activity in the samples. LC-MS/MS analysis revealed the parent compounds ETD and MBT, accounting for 3% and 2% of applied active ingredient in the soil layer, as well as dihydroxy-anilazine as the primary ANI metabolite. The results for ETD and MBT were matching with values obtained from samples of a 12 year old field plot experiment. The data demonstrate the long-term persistence of these pesticides in soils based on outdoor trials.

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1. Introduction

Worldwide, 2.3–2.6 million metric tons of pesticides were released into the environment for agricultural purposes between 1995 and 2007 (Aspelin, 1997; Aspelin and Grube, 1999; Donaldson et al., 2002; Kiely et al., 2004; Grube et al., 2011). Between 1987 and 1997, herbicides represented the major class of pesticides used in Germany. Herbicides accounted for 59.2% (21,520 tons) in 1987, decreasing to 47.5% (16,485 tons) in 1997. Within this time frame, urea herbicides were the largest fraction of all herbicides, accounting for 16.1–26.2% (Schmidt et al., 1999).

Pesticides containing the thiadiazolylurea herbicide ethidimuron (ETD; 1-(5-ethylsulfonyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea) were applied as non-selective herbicides for weed control on uncultivated land, railways, and in sugarcane plantations (Jarczyk, 1979; Kidd and James, 1994), and were applied in the Federal Republic of Germany (FRG) from 1975 until 1990. In the former German Democratic Republic (GDR), the application of ETD was not permitted (BVL, 2010).

The dimethylurea herbicide methabenzthiazuron (MBT; 1-(1,3-benzothiazol-2-yl)-1,3-dimethylurea) was used as a pre- and post-emergence selective herbicide to control annual grasses and

broad-leaved weeds during the cultivation of a large variety of field crops (beans, cereals, maize, potatoes and tree nurseries (Kidd and James, 1994)). The application of pesticides containing the active compound MBT was permitted in the FRG from 1971 until 1996, and in the area of the former GDR from 1972 until 1994 (BVL, 2010), and MBT-containing pesticides are still registered for use in Australia, India, and New Zealand (Kegley et al., 2010).

Fungicides represented the second largest pesticide class, accounting for 28.1% in 1987 and 27.1% in 1997 of all pesticides applied in FRG. The total mass of applied organic fungicides, including the active compound anilazine, amounted to 14.6% (1498 tons) in 1987 and 11.9% (1118 tons) in 1997 (Schmidt et al., 1999). The triazine fungicide anilazine (ANI; 4,6-dichloro-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine) was applied as a non-systemic foliar fungicide for various crops (vegetables, cereals, ornamental plants, fruits, coffee, tobacco, etc. (Kidd and James, 1994)). Pesticides containing the active compound ANI were used in the FRG from 1971 until 1996 (BVL, 2010). No data were found on the current use of ETD and ANI.

In 1987 only, areas of approx. 191,200 and 334,400 ha were treated with MBT and ANI, respectively, accounting for approx. 440 (MBT) and 640 (ANI) tons of applied active ingredients in the FRG (Gutsche and Strassemeyer, 2007). The amount of released ETD in the FRG accounted for approx. one ton in 1987, used for industrial purposes (BVL, 2012). Even though ETD and ANI are listed as obsolete and non-classified compounds as under pesticides in the WHO classification guidelines, MBT is listed as a slightly hazardous

* Corresponding author.

E-mail addresses: n.d.jablonowski@fz-juelich.de, n.d.jablonowski@daad-alumni.de (N.D. Jablonowski), p.burauel@fz-juelich.de (P. Burauel).

Table 1
Physico-chemical properties of all lysimeter soils originating from the MRZ field at the time of sampling in April 2006. All data are given in weight-%. \pm standard deviation of $n = 3$. For Al, Ca, Fe, K, Mg, Na values in bulk soil, the relative error is: for concentrations $>1\%$: $\pm 3\%$; $<0.1\%$: $\pm 20\%$; otherwise: $\pm 10\%$.

Soil	C_{org}	C_{inorg}	N	Al	Ca	Fe	K	Mg	Na
ETD soil	1.11 ± 0.01	0.065 ± 0.002	0.145 ± 0.003	3.7	0.44	1.7	1.7	0.28	0.74
MBT soil	1.21 ± 0.00	0.118 ± 0.008	0.155 ± 0.002	3.7	0.55	1.8	1.7	0.31	0.78
ANI soil	1.11 ± 0.00	0.059 ± 0.001	0.154 ± 0.004	3.7	0.49	1.8	1.6	0.30	0.71

compound (WHO, 2009). In the U.S. EPA Toxics Release Inventory List (TRI list) ANI is listed as a developmental and reproductive toxicant (USEPA, 2009).

Early studies revealed only a minor degradation capacity of MBT in soil (Cheng et al., 1978). A strong adsorption of this compound was demonstrated in soils, and shown to be highly correlated with the organic carbon content. This led to an accumulation of MBT in the surface soil (ploughing layer, 0–30 cm depth) with an insignificant leaching character (Kerpen and Schleser, 1977; Jarczyk, 1987). However, residues of aged and non-extractable MBT parent compound in soil were found to be bioavailable for maize plants (Kloskowski et al., 1986). Recent studies have shown that a translocation of MBT as well as ETD into deeper soil layers is also facilitated by preferential flow events (Kasteel et al., 2010). Compared to MBT, the adsorption affinity of ETD to soil is very low (Kerpen, 1978), resulting in a relatively high mobility in soil. As a consequence, ETD has been listed as a frequently detected pesticide in German groundwaters (LAWA, 2004). Anilazine has been found to form “bound residues” in the soil directly after application, mainly associated with different fractions of soil organic matter (Mittelstaedt et al., 1987). A stronger binding of ANI to the soil is given as a reason for a low microbial accessibility, compared to its main metabolite dihydroxy-anilazine, which is less strongly bound (Heitmann-Weber et al., 1994). To date, no experimental data on the long-term persistence of the pesticides ETD, MBT and ANI have been described.

The dynamics of pesticide fate in soils are difficult to simulate due to complex interactions of the pesticide in its surrounding matrix, including all biotic and abiotic influences, and due to the time restrictions of performed experiments. This study highlights data on the environmental long-term persistence of ^{14}C -labeled pesticides 9–17 years after application. Thus, our data provide important information on the fate of these chemicals in the environment.

2. Experimental section

2.1. Soils and pesticide application histories

For each pesticide compound, an individual outdoor lysimeter (surface area: 1 m^2) was used, containing an undisturbed soil column (soil depth: 1.1 m) of an Orthic Luvisol (C_{org} : 1.2%; sand: 6.4%; silt: 78.2%; clay: 15.4%; pH: 7.2) from Jülich-Merzenhausen (MRZ), Germany. Detailed properties of the lysimeter soils used are given in Table 1.

ETD was applied as [thiadiazol-2- $^{13}/^{14}\text{C}$] ethidimuron in November 1997, with a total of 1.23 kg active ingredient (a.i.) ha^{-1} , and a radiochemical purity of $>98.0\%$.

Table 2
Application time and total quantities of ETD, MBT and ANI on lysimeter and field soils. a.i. active ingredient; *in total after three applications, 1988, 1992, and 1994; **in total after 5 consecutive annual applications, 1985–1989. Sampling of all lysimeter soils was performed in April 2006. Information on MRZ field soil was taken from Kasteel et al. (2010). MRZ field soil was collected in November 2009 from 0 to 30 cm depth.

Soil	Date of application [dd/mm/yyyy]	Applied a.i. [mg m^{-2}]	Total applied a.i. [kg ha^{-1}]	Applied ^{14}C activity [MBq m^{-2}]	Specific ^{14}C activity [kBq mg^{-1}]
ETD soil	13.11.1997	123.3	1.23	107.3	870.0
MBT soil	29.11.1994 (last application)	879.0*	8.79*	261.6*	297.7
ANI soil	13.06.1989 (last application)	1978**	19.8**	372.5**	188.4
MRZ field: ETD	13.11.1997	118.0	1.18	—	—
MRZ field: MBT	13.11.1997	130.0	1.30	—	—

Three applications of [phenyl- ^{14}C] methabenzthiazuron (radiochemical purity: $>99.9\%$) were performed in November 1988, 1992, and 1994, with a total application amount of $8.79 \text{ kg a.i. ha}^{-1}$ (Pütz, 1993).

ANI was applied directly on the soil as [benzene-ring- ^{14}C] anilazine (radiochemical purity: 98%) in June in five consecutive years (1985–1989), with an annual application amount of $4 \text{ kg a.i. ha}^{-1}$ (Heitmann-Weber et al., 1994; Mittelstaedt and Führ, 1996). This application amount was directed especially for research purposes, exceeding the amount normally used in accordance with good agricultural practice. The goal for this application quantity was the production of a high concentration of non-extractable residues in order to be able to characterize binding mechanisms of ANI and its metabolites in soil organic matter pools using NMR spectroscopical methods (Wais et al., 1995).

To validate and compare field-lysimeter and real field data on pesticide fate, the soil from a previous field study in MRZ ($50^\circ 54' \text{ N}$; $6^\circ 24' \text{ E}$, according to the geographic coordinate system WGS 84 (Kasteel et al., 2010), absolute altitude approx. 93 m), using non- ^{14}C -labeled ETD and MBT was additionally collected (0–30 cm depth; MRZ field soil).

The time and amount of applied pesticides and associated ^{14}C activity is summarized in Table 2. All pesticides were uniformly applied on the soil surfaces but were mixed in the upper soil layer 0–30 cm due to ploughing in the subsequent years. Details of the chemical properties and molecular structure of the used pesticides are given in Table 3 and Fig. 1.

2.2. Sampling and determination of ^{14}C activity

The lysimeter soils containing the long-term aged pesticide residues were sampled 9 (ETD), 12 (MBT), and 17 (ANI) years after application. Multiple soil samples were randomly collected from each lysimeter (approx. 3 kg), using a stainless steel Humax soil core sampler with a diameter of 3 cm. MBT and ANI soil samples were taken from the layer at a depth of 0–30 cm, since no significant difference of residual ^{14}C -pesticide distribution was expected in the upper 30 cm of the soil due to regular annual ploughing. Since soil in the ETD lysimeter was not ploughed during the 5 years prior to sampling, the soil cores were subdivided into 0–10, 10–20, and 20–30 cm soil layers. In our experiment, only soil from the 0–10 cm layer was used, since we detected highest residual ^{14}C activity in this layer. All soil cores of each individual lysimeter soil were air-dried to a residual water content of 7–12%, 2 mm sieved, homogenized, and stored in the dark at 3°C until further analyses. To determine residual ^{14}C -activity, a sample of 100 g of each individual soil was dried at 105°C to accelerate the experimental process, and was ground in a mortar. Five subsamples (0.5 g) of each homogenized soil were used for combustion (Biological Oxidizer OX500; R.J. Harvey Instrument Corporation). The emerging $^{14}\text{CO}_2$ was trapped in Oxy-solve C-400 scintillation cocktail (Zinser Analytik), and the radioactivity was detected by a liquid scintillation counter with internal standard (LSC; 2500 TR, Tri-Carb, Packard Liquid Scintillation Analyzer). For all pesticide residues, the drying temperature had no effect on the residual ^{14}C activity, since no differences were observed when additional soil samples were air-dried only prior combustion. Due to different soil management, we used an estimated bulk soil density of 1.5 g cm^{-3} (ETD soil, since no ploughing was conducted for 5 years prior to sampling, and in accordance with (Kasteel et al., 2010)), and 1.3 g cm^{-3} (ANI soil and MBT soil; in accordance with OECD guideline 307 (OECD/OCDE 307, 2002) and due to previous ploughing) for all calculations. MRZ field samples were collected from a depth of 0–30 cm, 12 years after the experimental field application of ETD and MBT (Table 2).

Table 3
Chemical properties of ETD, MBT and ANI. Information taken from (Kidd and James, 1994).

	Molecular formula	Chemical family	Molecular weight [g Mol ⁻¹]	Melting point [°C]	Solubility in acetone/water* [g L ⁻¹ or **kg ⁻¹ at 20 °C]
ETD	C ₇ H ₁₂ N ₄ O ₃ S ₂	urea; thiadiazole	264.33	155.9–156	150.0**/3.0*
MBT	C ₁₀ H ₁₁ N ₃ OS	urea; benzothiazole	221.29	119–121	115.9/0.059*
ANI	C ₉ H ₅ Cl ₃ N ₄	triazine; organochlorine	275.54	159–160	100.0/0.008*

2.3. Accelerated solvent extraction of the soil samples

A maximum yield of all aged pesticide residues from the soils was achieved by applying an accelerated solvent extraction (ASE; ASE 200, Dionex) with acetone–water solution (4 + 1, v + v) as a solvent. The sample preparation and ASE setting was in accordance with a previous study (Jablonowski et al., 2009), slightly modified with regard to the melting points of the respective pesticides (Table 3), as described in the following. For each individual soil, triplicates of 10 g dried and homogenized soil of each lysimeter were weighed into 11 mL stainless steel ASE cells. For soil samples containing the long-term aged ¹⁴C-labeled ETD and ANI residues, an extraction temperature of 135 °C was applied. For ¹⁴C-labeled MBT residues, the extraction temperature was adjusted to 105 °C. The preheating time was 5 min, and the static time 15 min. All samples were extracted at 100 bar (1500 psi) with a flush volume of 60% of the cell volume and a purge time of 100 s. Each sample was extracted eight consecutive times under the same ASE conditions. Triplicates of 1 mL of each ASE extract containing the extracted residual ¹⁴C activity and pesticide residues were mixed with 3.5 mL scintillation cocktail (Instant Scint-Gel Plus™, Perkin–Elmer), and ¹⁴C activity was determined by LSC using an external standard for quenching correction. Parallels of MRZ field soil samples were treated in accordance with the ETD and MBT soils extraction procedure, and extracts were used for residue determination using LC-MS/MS.

2.4. LC analysis of ASE extracts

2.4.1. Radio-HPLC analysis

To obtain at first qualitative information on the nature of the ¹⁴C activity in the extracts of ¹⁴C-labeled ETD, MBT, and ANI treated soil, triplicate samples were firstly subjected to radio-HPLC (Berthold Radio-Flow Detector LB 590, Jasco UVD 2075 detector, solid scintillation cell YG 150 U4, pump 1580, GINA 50 sampler). All triplicate samples of the first extracts were concentrated to a final volume of 0.8–1.8 mL by vacuum evaporation using a Büchi Syncore® (gradient: 45–55 °C, vacuum 550–70 mbar, 120 rpm, over a total time of approximately 6 h; Büchi Vacuum Pump Vac® V-500, vacuum Controller V-805). The concentrated samples were transferred to 2 mL Eppendorf® cups and centrifuged for sedimentation of the particles for 15 min at 15,000 g (Hettich Mikro Rapid). The injection volume of the concentrated samples was 250 µL at a flow rate of 1 mL min⁻¹. The applied HPLC column was a Synergy Fusion RP 80A (250 mm × 4.6 mm × 4 µm, Phenomenex, Germany), with an additional pre-column (LiChrospher 60 RP-select B, 4 mm × 4 mm, 5 µm, Merck, Germany). The eluent used (pH 3.5) was prepared using 490 mL dionized water (Milli-Q Plus 185, Millipore purification system), 10 mL methanol (HPLC-grade, Merck, Germany), and 0.5 mL concentrated H₃PO₄ (25%, Grüssing, Germany). The UV detector wave length was 225 nm for ETD, MBT, and A-MBT. The applied gradient conditions were at time 0–6 min 100% acidified eluent (pH 3.5), at time 23 min 43% acidified eluent/57% methanol, at time 28 and 32 min 100% methanol, and at time 42 min until the end of the gradient 100% acidified eluent. The detection limit was 40 Bq mL⁻¹.

2.4.2. LC-APCI-MS-MS analysis

Prior to analysis, all sample ASE extracts were vacuum filtered using glass fiber filters (pore size: ≥1 µm, 47 mm/No.6, Schleicher & Schüll, Germany) to remove particulates in the extracts.

2.4.2.1. Reagents and material. Ethidimurone (ETD), methabenzthiazurone (MBT), anilazine (ANI), dihydroxyanilazine (di-OH-anilazine) and dimethoxyanilazine

(di-OMe-anilazine) were donated by Bayer Crop Science AG (Monheim, Germany). Isoprotrurone (IPU) and d₅-2-hydroxyatrazine purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) were used as internal standards (IStd) for the quantification of ETD, MBT and ANI, dihydroxyanilazine, dimethoxyanilazine, respectively. Ammoniumacetate (Fractopur), formic acid (100%, Suprapur) and acetonitrile (LiChrosolv) were obtained from Merck (Darmstadt, Germany).

2.4.2.2. LC-APCI-MS-MS. Liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-MS-MS) was performed with an Agilent 1100 series HPLC (Santa Clara, CA, USA) equipped with a binary pump, a thermostated column and a HTC Pal Autosampler (CTC Analytics, Zwingen, Switzerland) and coupled to a Thermo Electron (Waltham, MA, USA) TSQ Quantum triple quadrupole mass spectrometer, working in the multiple reaction monitoring mode (MRM). LC separations were carried out with a Phenomenex Synergi 4µ Polar RP column, 150 × 3.0 mm I.D. (Torrance, CA, USA) at 25 °C and 0.5 mL min⁻¹ as flow rate. For the ETD and MBT determination, 1 mM ammoniumacetate + 0.1% formic acid as solvent A and acetonitrile + 0.1% formic acid as solvent B were applied with the following gradient: starting with 30% B for 2 min, increasing to 70% B within 3 min and kept constant for 1 min. Within 1 min, it was changed back to the initial composition and kept constant for 8 min. For the detection of ANI and its metabolites, 5 mM ammoniumacetate as solvent A and acetonitrile as solvent B were used with the following gradient: starting with 100% A for 6 min, it was changed to 100% B within 3 min and kept for 4 min. Within 2 min, it was changed to the initial composition and kept constant for 5 min. The mass spectrometer was operated in the positive APCI mode for the quantification of ETD and MBT, using isoprotrurone (IPU) as internal standard, and in the negative APCI mode for ANI and its metabolites using d₅-2-hydroxyatrazine as internal standard. The vaporizer and capillary temperature were set to 400 and 300 °C, respectively. The discharge current was 3.0 µA. MS–MS parameters of the analytes were determined first by flow injection analysis (FIA) of standard solutions using the inbuilt syringe pump, listed in Table 4. Argon was used as a collision gas (1.5 mTorr).

2.4.2.3. Calibration and quantification. Stock solutions (0.5 g L⁻¹) of the individual analytes were obtained by dissolving the compounds in acetone–water (4 + 1, v + v), in accordance with the ASE extraction solution. For LC-MS quantification of ETD and MBT, standards (MBT: 0.2–200 µg L⁻¹, ETD: 5–25 µg L⁻¹) were freshly prepared by diluting the stock solution, containing 165 µg L⁻¹ IPU as the internal standard. These standards were used to generate the response factors *f_i* in relation to the internal standard. Each ETD and MBT soil extracts were spiked with 10 µL IPU (30 µg L⁻¹). Quantification of ETD and MBT was conducted using the previously determined response factors *f_i*. Limits of quantitation were determined at 0.27 µg L⁻¹ for ETD and 0.2 µg L⁻¹ for MBT, respectively. For LC-MS quantification of ANI metabolites (ANI as the parent compound was not detected in any sample), two proper standards (di-OH-anilazine: 5–500 µg L⁻¹, di-OMe-anilazine: 11–110 µg L⁻¹) were freshly prepared by diluting the stock solutions containing 500 µg L⁻¹ d₅-2-hydroxyatrazine as the internal standard. Limits of quantitation were determined at 5 µg L⁻¹ for di-OH-anilazine and 11 µg L⁻¹ for di-OMe-anilazine, respectively.

2.5. Elemental analysis of solid samples

Elemental analysis was performed in triplicates as described elsewhere (Jablonowski et al., 2009). Briefly, homogenized subsamples were dried at 105 °C, and carbon was determined by radiofrequency heating in flowing oxygen and

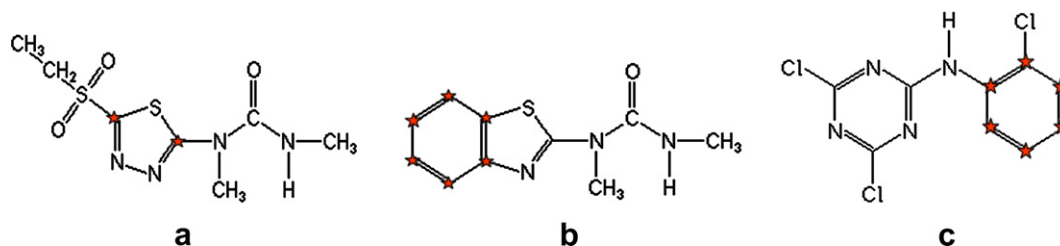


Fig. 1. Molecular structure of (a) ETD, (b) MBT, (c) ANI. ★ = ¹⁴C-labeling position.

Table 4

Precursor and product ions for LC-APCI-MS-MS analysis of MBT, ETD, IPU (internal standard), and ANI, di-OH-anilazine, di-OMe-anilazine and d₅-2-hydroxyatrazine (internal standard) with their optimized values for collision energy and tube lens.

Compound	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy [eV]	Tube lens [V]
ETD	265	208	20	100
MBT	222	165	22	107
IPU	207	72	36	100
ANI	273	35	24	90
Di-OH-anilazine	237	151	28	50
Di-OMe-anilazine	265	192	50	60
D ₅ -2-hydroxyatrazine	201	83	30	65

subsequent infrared detection by a Leco RC-412 multiphase carbon determinator. Nitrogen was determined by thermal conductivity detection using a Leco TCH 600. For elemental (Al, Ca, Fe, K, Mg, Na) analysis of the soils, 50 mg of dried sample was decomposed with a mixture of 0.25 g of lithium-borate for 30 min at 1000 °C in a muffle furnace. The flux was dissolved in 30 mL HCl (5%; 0.95 M, respectively) and adjusted to a total volume of 50 mL. The analysis was performed using inductively coupled plasma with optical emission spectroscopy (ICP-OES; TJA-IRIS-Intrepid spectrometer, Thermo).

2.6. Calculations

An exponential decay function was employed to calculate the half-lives for ETD and MBT,

$$T_{1/2} = \frac{\ln 2}{\ln(N_0/N)}t,$$

where N_0 is the initial amount and N the measured amount of the respective substance after time t . Since ANI was not detected as the parent compound, a half-life calculation was not possible.

2.7. Additional experimental parameters

Climate data were collectively surveyed for all presented pesticide aging studies by the Research Centre Jülich, Germany (Table 6) (FZJ, 2012). Data on management and crop rotation for the used soils are presented in Table 7.

3. Results and discussion

3.1. Analysis of residual ¹⁴C activity in soil samples

Values of determined residual ¹⁴C activity in the soil samples are presented in Table 5 and varied by the chemical property of the respective ¹⁴C-labeled pesticide. It has been demonstrated previously that ETD has greater mobility, and adsorption was significantly correlated with C_{org} , N, and cationic exchange capacity in soils (Kerpen, 1978). The adsorption of ETD was found to be very low in soils with a C_{org} content of 1.1% (Kerpen, 1978), as was the case for the soils used in this study (Table 1). With the lowest environmental aging time of 9 years for ETD, the calculated residual

Table 6

Meteorological data for the lysimeter soils (ETD-, MBT-, and ANI soil) and the MRZ field soil were collectively surveyed for the time frame of the presented pesticide aging studies (1985–2006). Detailed daily climate data are freely available for the time period 1961–2010 on the web-pages of the Research Centre Jülich, Germany (FZJ, 2012).

Year	Temperature annual mean [°C]	Temperature annual maxima [°C]	Temperature annual minima [°C]	Relative humidity [%]	Precipitation amount [mm]
1985	8.6	31.6	−17.5	79	637
1986	9.3	35.9	−16.9	77	676
1987	8.9	31.7	−16.4	80	793
1988	10.4	29.7	−8.1	80	773
1989	10.8	32.8	−6.6	78	620
1990	10.8	35.6	−4.1	77	701
1991	9.6	33.3	−13.2	78	634
1992	10.5	33.0	−8.1	80	676
1993	9.9	30.0	−9.3	79	781
1994	11.1	36.0	−11.1	79	611
1995	10.7	35.0	−8.4	79	663
1996	8.8	32.0	−13.5	76	535
1997	10.1	33.3	−20.4	77	679
1998	10.3	33.6	−11.1	79	795
1999	10.8	31.8	−9.7	78	723
2000	10.9	34.2	−6.5	80	779
2001	10.4	35.9	−8.4	79	801
2002	10.7	34.6	−7.3	79	829
2003	10.6	37.6	−13.7	75	560
2004	10.2	32.9	−8.7	80	668
2005	10.7	33.0	−9.8	78	653
2006	11.0	36.5	−10.2	79	723
2007	11.1	34.9	−7.6	79	878
2008	10.5	31.5	−6.5	78	725
2009	10.5	35.2	−18.6	79	728
Mean	10.3	33.6	−10.9	79	706

¹⁴C activity in ETD soil accounted for 19% of total ¹⁴C activity applied in the analyzed soil layer at 0–10 cm (Table 5). However, analyses of the depths of 10–20 cm and 20–30 cm revealed only a slightly decreased amount of 16% and 12% of total ¹⁴C activity applied. The relatively even distribution of residual ¹⁴C activity can be attributed to previous ploughing of the lysimeter soil (ploughing depth: 0–30 cm). Considering the residual ¹⁴C activity of these two layers, the total residual ¹⁴C activity for 0–30 cm depth accounts for 47% of the total initially applied ¹⁴C activity. Therefore, the detection of these ¹⁴C values in the upper ETD soil layer after environmental long-term aging is surprising. However, recent findings regarding the persistence of ETD in soil support this outcome, indicating the long-term persistence of this compound in soil (Kasteel et al., 2010).

The residual ¹⁴C activity in MBT soil accounted for 35% of the total applied in the soil layer at a depth of 0–30 cm (Table 5). Since MBT was found to be partly biodegradable (Printz et al., 1995; Malouki et al., 2003; Del Pilar Castillo and Torstensson, 2007), it can be assumed that the detected residual ¹⁴C activity is associated with

Table 5

Total residual ¹⁴C activity per soil layer and total extracted ¹⁴C activity and parent compound after eight consecutive extraction steps in ETD, MBT, and ANI soils. Quantified ETD, MBT, and di-OH-anilazine* by means of ASE extraction and LC-MS/MS analyses. d. detected. ±standard deviation of $n = 3$ –9.

Soil(soil layer in cm)	Aging time [years]	Residual ¹⁴ C activity of total applied in soil layer [%]	Total extractable of residual ¹⁴ C in samples [%]	Concentration parent compound extractable in soil [μg kg ^{−1}]	Parent compound of initially applied calculated for m ² and soil layer [%]	Predicted parent compound of total initially applied based on regulatory information [ng]–[%]
ETD soil (0–10)	9	18.7	89.8 ± 3.0	24.5 ± 2.0	3.0	0.7–5.7 · 10 ^{−7}
MBT soil (0–30)	12	34.8	26.0 ± 0.3	45.9 ± 8.3	2.0	0.1–1.7 · 10 ^{−8}
ANI soil (0–30)	17	43.2	41.5 ± 0.4	—	—	—
ANI soil: di-OH-anilazine	—	—	—	248.8 ± 10.8*	4.9 ^a	—
ANI soil: di-OMe-anilazine	—	—	—	d.	—	—
MRZ field (0–30): ETD	12	—	—	12.5 ± 1.0	3.8	0.7–5.7 · 10 ^{−7}
MRZ field (0–30): MBT	12	—	—	5.7 ± 0.4	1.9	6.2–4.7 · 10 ^{−6}

^a Calculated parent compound equivalents.

Table 7

Soil management data for the lysimeter soils (ETD-, MBT, and ANI soil) and the MRZ field soil for the time frame of the initial study. – indicates time before initiation of the study, i.e. pesticide application. Cultivation: all measures including fertilization and plant protection were closely coordinated with agricultural practice. Date indicates the year of harvesting. b.s. bare soil, i.e. no crop cultivation. *Year of sampling of the individual soil.

Year	ETD soil	MBT soil	ANI soil	MRZ field
1985	–	–	winter wheat	–
1986	–	–	winter barley	–
1987	–	–	winter rye	–
1988	–	winter wheat	oat	–
1989	–	winter barley	winter wheat	–
1990	–	oat	winter barley	–
1991	–	sugar beet	rye	–
1992	–	b.s.	sugar beet	–
1993	–	winter wheat	winter wheat	–
1994	–	sugar beet	oat	–
1995	–	winter wheat	b.s.	–
1996	–	corn	winter wheat	–
1997	b.s.	oat	winter barley	b.s.
1998	b.s.	winter wheat	winter wheat	b.s.
1999	b.s.	winter wheat	winter wheat	b.s.
2000	b.s.	oat	sugar beet	b.s.
2001	b.s.	winter wheat	winter wheat	b.s.
2002	b.s.	sugar beet	winter wheat	b.s.
2003	b.s.	sugar beet	b.s.	winter barley
2004	b.s.	winter wheat	winter barley	winter wheat
2005	b.s.	winter wheat	b.s.	sugar beet
2006	b.s.*	sugar beet*	sugar beet*	winter wheat
2007				winter barley
2008				rape
2009				winter wheat*

both the parent compound as well as metabolites and nonspecific ^{14}C residues incorporated into microbial biomass. Interestingly, the amount of residual ^{14}C activity recovered after 12 years of environmental aging is in the same range as previously described after an environmental aging time of 4 years, accounting for 35–40% (Brumhard, 1991). This indicates a stabilization and persistence of this compound in the analyzed soil layer even on longer time scales. The adsorption of MBT in soils was found to be strongly associated with the organic carbon content (Raman, 1987; Baez et al., 2001; Brumhard, 1991). A strong adsorption or even entrapment of the pesticide molecules as part of a soil-bound residue fraction may result in an exclusion of the molecule from microbial attack (Printz et al., 1995). However, leaching below a depth of 30 cm has been reported to account for 9% of total applied MBT ^{14}C activity after 4 years in the same soil as used for our experiments (Brumhard, 1991). The C_{org} content was relatively low at 1.2% in MBT soil (Table 1) but in a normal range for agricultural soils in Germany. Considering the C_{org} as the main binding site for MBT and the limited biodegradability of MBT, the presence of 35% of the total applied ^{14}C activity in the soil layer after a total aging time of 12 years provides novel information on the environmental behavior and turnover of this herbicide as a representative of urea benzo-thiazole pesticide.

The residual ^{14}C activity in the ANI soil accounted for 43%, calculated for a depth of 0–30 cm. Considering the environmental aging time of 17 years, the detected amount of residual ANI ^{14}C activity could be regarded as relatively high. This outcome is somewhat surprising demonstrating a strong adsorption of ANI to soils, predominantly in the soil organic matter, and an exclusion of the molecule from potential microbial degradation (Mittelstaedt et al., 1987; Heitmann-Weber et al., 1994; Wais, 1997).

Overall, the observation that a major fraction of the residual pesticide ^{14}C activity is located in the upper soil layers agrees with previous studies dealing with other pesticides aged in outdoor field

lysimeters (Buraue and Führ, 2000; Jablonowski et al., 2009). At this point it remains unknown to which extent the detected non-extractable residual ^{14}C activity of the individual pesticide is associated with the parent compound, its metabolites, or is sequestered into the soil microbial biomass or the organic carbon fraction of the soil itself.

3.2. Analysis of ASE soil extracts

3.2.1. ^{14}C analyses

For all ^{14}C -labeled pesticide residues, the extraction efficiency was highest in the first ASE extraction step, as shown in Fig. 2. In total, the extracted ^{14}C activity accounted for 90, 26, and 41% of the residual ^{14}C activity in the ETD soil, MBT soil, and ANI soil, respectively, after eight consecutive extractions (Table 5). Since the extraction method used in our study yielded higher ^{14}C activity as compared to the study by Brumhard after a much shorter aging time (Brumhard, 1991), our findings prove that ^{14}C MBT residues remain stable and strongly bound to the soil even after long-term aging. However, compared to other literature data (Kasteel et al., 2010; Lagarde et al., 2006), the extraction efficiency of the ^{14}C activity for ETD and MBT was much lower for ^{14}C MBT soil residues, which must in turn be attributed to the longer environmental aging times and the different extraction procedure used here compared to the cited studies.

The ^{14}C values in the extracts from the ANI soil in this study were higher, even after much longer incubation periods, compared with previous data (Mittelstaedt et al., 1987; Heitmann-Weber et al., 1994). The higher value of extractable ^{14}C activity accounting for 41% of total ^{14}C activity in the sample must be explained by the more efficient ASE extraction method applied for this compound. It should be considered that the higher amount of extractable ^{14}C activity could also be related to unspecific ^{14}C residues, which might be more extractable than the parent compound or its metabolites itself. However, since microbial mineralization of ANI was found to occur only to a minor extent, it cannot be excluded that the non-extracted residual ^{14}C activity is associated with the parent compound ANI or with its metabolites, such as di-OH-anilazine. This residual ANI ^{14}C fraction is most likely associated with the soil organic carbon such as humic acids, which play an important role in the bonding of ANI (Mittelstaedt et al., 1987; Heitmann-Weber et al., 1994; Wais et al., 1995).

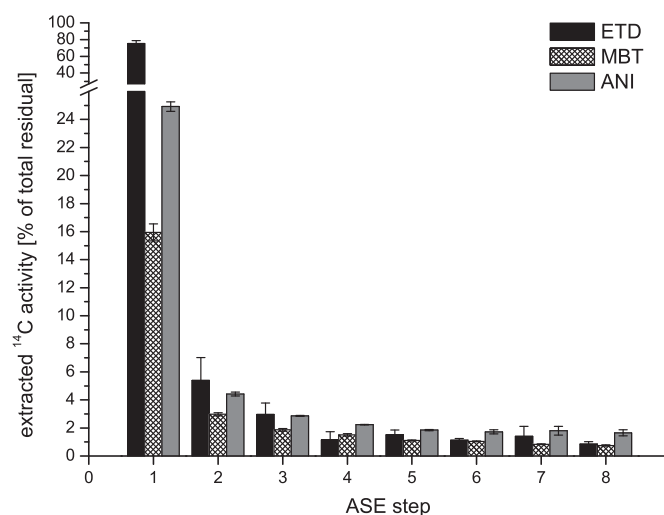


Fig. 2. Detected ^{14}C activity by means of LSC in ASE extracts of ETD, MBT, and ANI soils.

3.2.2. Radio-HPLC analysis of concentrated ASE soil extracts

The recovery of ^{14}C activity in the concentrated ASE soil extracts for radio-HPLC analyses accounted for $73.4\% \pm 1.0$ (ETD soil), $78.8\% \pm 5.6$ (MBT soil), and $59.5\% \pm 0.5$ (ANI soil), respectively. Losses in the concentration process occurred to a minor extent in the form of solid material (ETD soil extract: $1.7\% \pm 0.1$; MBT soil extract: $4.5\% \pm 1.5$; ANI soil extract: $1.2\% \pm 0.6$). This was separated by centrifugation of the concentrates, but no ^{14}C activity was found in the condensate. Radio-HPLC of the concentrated ETD soil extract revealed the parent compound ETD, as well as two unidentified metabolites, whereby no ETD metabolite standards were available. However, previous studies described A-ETD as a major ETD metabolite in soils (Jarczyk, 1979; Lagarde et al., 2006; Kasteel et al., 2010), which might be represented by one of the unidentified metabolites. Analyses of the concentrated MBT soil extracts showed no clear peaks, neither for MBT, nor any identifiable metabolite. The fact that radio-HPLC analyses of the MBT soil extract revealed no visible peaks must be attributed to the generally lower applied ^{14}C activity and lower ^{14}C activity in this soil extract concentrate, which are distributed in a number of unspecific peaks (data not shown). This observation can also be explained by the degradability of MBT in soils, resulting, however, in very small metabolite fractions (Cheng et al., 1978; Mittelstaedt et al., 1977). Concentrated extracts of ANI soils revealed minor but definite peaks for the main ANI metabolite di-OH-anilazine (data not shown), supporting the finding of di-OH-anilazine using LC-MS/MS analyses.

3.2.3. LC-MS/MS analysis

In accordance with the highest amount of extracted residual ^{14}C activity in the first ASE extracts, a major fraction of the pesticide parent compound was detected for ETD and MBT (Fig. 3). As presented in Table 5, the total extractable and detectable amount of parent compound accounted for 24.5, and $45.9 \mu\text{g kg}^{-1}$ for ETD and MBT, respectively. These values represent 3.0% for ETD and 2.0% for MBT of the total parent compound initially applied in the analyzed soil layers at depths of 0–10 cm (ETD soil) and 0–30 cm (MBT soil), respectively. Due to this relatively small fraction of the parent compounds ETD and MBT, a combination of translocation to deeper soil layers by leaching, degradation to metabolites, mineralization and plant uptake must be assumed (Kloskowski and Führ, 1987). Due to the very low vapor pressure of ETD and MBT (ETD: $<0.001 \text{ mPa}$, MBT: approx. 590 nPa , both at 20°C ; (Kidd and James,

1994)), a loss of the compounds by volatilization after soil application is rather unlikely.

To validate the data obtained in the ETD and MBT outdoor long-term lysimeter studies, we compared the data with samples from a field trial with ETD and MBT. This field study was started at the same time as the application of ^{14}C ETD on the respective lysimeter (Table 2). As shown in Table 5, the percentage of extractable parent compound accounted for 3.8% for ETD and 1.9% for MBT of the total initially applied in MRZ field soil. These values are of the same magnitude as the values obtained from the lysimeter soils, even though the field samples were environmentally aged for three additional years compared to the lysimeter soil samples. These values clearly demonstrate the persistence of ETD and MBT in the analyzed soil layers and the validity of pesticide fate data received from outdoor lysimeter studies.

The field fate half-lives have been previously calculated based on data from the same field experiment but after a much shorter aging time of approximately 2 years (ETD: 870 d; MBT: 30 d (Kasteel et al., 2010)). The following parameters were considered for calculation: the respective field soil density (MRZ field soil: 1.3 g cm^{-3} ; ETD soil: 1.5 g cm^{-3}), the soil layer (MRZ field soil: 0–30 cm depth; ETD soil: 0–10 cm depth) and pesticide aging time (MRZ field soil: 12 years; ETD soil: 9 years). The resulting field half-life for ETD accounted for 925 d in the field after 12 years. In the lysimeter, the half-life accounted for 648 d after 9 years of environmental aging, considering only the extractable ETD residues in the soil layer at 0–10 cm. Assuming a similar ETD distribution in the lysimeter soil for the layer at a depth of 0–30 cm, the calculated half-life accounted for 943 d. Based on our mass recovery calculations, the values obtained for the herbicide ETD are therefore in accordance with the previously calculated half-lives after environmental aging, as presented by Kasteel et al. (Kasteel et al., 2010). However, the calculated half-life for ETD in our long-term study is almost eight times higher when compared to a previously published half-life for this pesticide of 3–4 months (Kidd and James, 1994). Based on our results, the half-life for ETD in soil is approximately 2.6 years.

The calculated half-lives for MBT account for 765 d in the MRZ field soil, and 778 d in the MBT soil. On average, these half-lives exceeded the calculated environmental half-life by Kasteel et al. (Kasteel et al., 2010) by a factor of 26. However, our half-lives for MBT are approximately six times higher when compared to a previously given half-life in soil accounting for 135 days (University of Hertfordshire, 2011). According to our results, the calculated half-life for MBT accounts for approximately two years. Even though the environmental half-life for ETD could be confirmed when compared to the study by Kasteel et al., the much higher half-lives for MBT clearly demonstrate the complexity of environmental mechanisms that influence the fate of pesticides. As has been demonstrated in previous studies on the s-triazine herbicide atrazine, simplified half-life calculations based on short-term experiments can be misleading since much higher atrazine concentrations than expected were found after long-term environmental aging (Jablonowski et al., 2009, 2010, 2011).

Even though the parent compound ANI was not detected in the soil extracts, its main metabolite di-OH-anilazine, as well as di-OMe-anilazine were detected. The quantified value for di-OH-anilazine accounted for $248.8 \mu\text{g kg}^{-1}$, whereas the value for di-OMe-anilazine was not in the linear range for quantification (estimated in a low μg -range). These values accounted for 4.9% and approximately 0.1%, calculated on a mass basis of total parent compound applied (Table 5). However, since only 41% of residual pesticide ^{14}C could be extracted, and since ANI was found to form strong soil-bound residues to a large extent (Mittelstaedt et al., 1987; Haider et al., 1993; Wais, 1997), we assume that the non-extractable

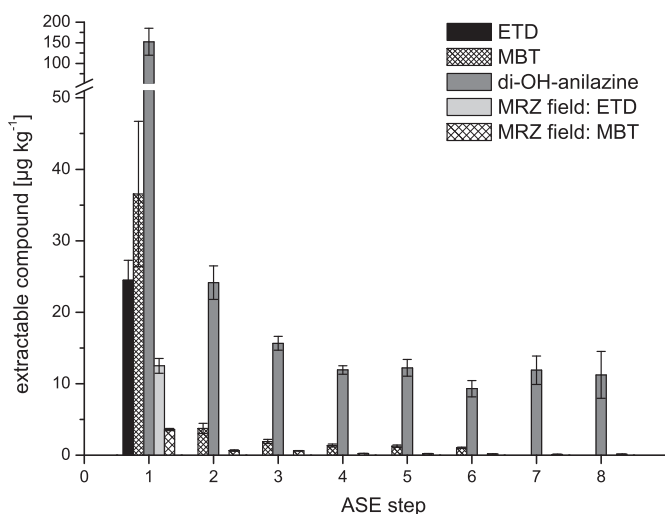


Fig. 3. Detected pesticide residues by means of LC-MS/MS analyses in ASE extracts of ETD, MBT, and ANI soils.

fraction likely consisted of the parent compound ANI or of a more recalcitrant fraction of its metabolites. Since other comparable data on the long-term fate of these pesticides are lacking, the magnitude of the residual pesticide fractions is difficult to assess and to classify. However, the total amount of predicted parent compound of total initially applied based on regulatory information approaches zero (Table 5).

3.3. Climate and crop rotation

Climate and crop rotation may influence the fate and turnover of pesticides. For all investigated soils, climate and crop rotation data were monitored and are presented in Tables 6 and 7, respectively. Climate data, such as temperature and precipitation were equal for all experimental soils since all experiments were conducted in the same area. Crop rotation was in accordance with good agricultural practice and was very similar to all soils, except for ETD soil (bare soil only, no crop rotation; Table 7). As described above (chapter 3.2.3), the residual amount of extractable ETD from the lysimeter soil accounted for 3.0% of total applied after 9 years in the soil layer 0–10 cm depth (Table 5). In comparison, the amount of extractable ETD from the field soil accounted for 3.8% of total applied after 12 years in the soil layer 0–30 cm depth (Table 5). Considering that this value was calculated for a soil layer of 0–30 cm depth and after a longer environmental aging time of three additional years, this value is in line with the value obtained after 9 years in the soil layer 0–10 cm from the lysimeter ETD soil, managed without crop rotation.

The values of extractable MBT from the MBT soil and the MBT field soil accounted for 2.0% and 1.9% (Table 5), respectively, and were therefore in the same range, irrespective of differences in crop rotation (Table 7). In the short-term directly after pesticide application, we would assume that climate and crop rotation may have significant effects on pesticide fate, and must therefore be considered. In long-term studies such as ours where pesticides had aged under outdoor conditions for 9–17 years, it can be assumed that an equilibrium of sorbed or entrapped pesticide residues had been established in the soils, irrespective of the recent soil management. However, as suggested in an additional study on the investigated pesticides, soil drying and wetting leads to a release of significant higher amounts of pesticide residues into solution compared to a constantly moistened soil (Jablonowski et al., 2012). Therefore, these long-term aged soil-associated pesticide residues are most likely not excluded from environmental influences. However, data on risk assessment of these extractable pesticide residues are missing at this point.

The influence of crop rotation and climate on the fate and turnover of ANI remains speculative at this point since comparable data on the long-term fate of ANI or metabolites are not available. However, even an almost constant cultivation of the ANI soil (Table 7) did not cause a complete dissipation of the ANI residues, as indicated by the quantified residual ^{14}C activity and the main metabolite di-OH-anilazine still present in this soil.

3.4. Conclusion

This study demonstrates the environmental long-term persistence of three different pesticides ETD, MBT and ANI, as well as their metabolites in soils. Our results are in disagreement with literature data, exceeding the given half-lives for these compounds in soils by far. The observed persistence is likely to be valid for a number of other pesticide compounds regularly applied to soils, as previously described for e.g. atrazine and its metabolites (Jablonowski et al., 2008, 2010, 2011). There is common agreement that the environmental aging of pesticides strongly affects the

diffusion and stabilization of the chemical molecules in soil micropores, soil aggregates and/or organic matter. This is also likely in the case of biodegradable molecules, which are excluded from microbial attack, persisting in soils for decades (Jablonowski et al., 2010). Most of the available data on pesticide fate in natural systems are based on short-term experiments or in situ observations which cannot consider long-term environmental influences on the chemical or pathways of its dissipation due to a lack of information on application time and quantities. Due to regular pesticide applications in agricultural practice, the pesticide burden in soils could be considerable. The potential toxicity of chemical residues in soils is controversially discussed with regard to their environmental aging time (Alexander, 2000). Our presented and previous findings on atrazine demonstrate the long-term persistence of various, formerly applied pesticide compounds in soils, irrespective of their potential residual toxicity (Jablonowski et al., 2008, 2010, 2011, 2012). In our studies, the obtained data are related to a limited soil horizon only (0–30 cm depth), and do not consider residues that could be present in deeper layers, leachate or pesticide loss by other forms of dissipation. Therefore, our results provide an estimation of the overall persistence of the chemicals investigated in the soil environment, based on their mass recovery with the methods applied. The persistence and magnitude of pesticide non-extractable residue formation in soils were found to be mainly compound specific (Barriuso et al., 2008; Burael and Führ, 2000; Burael and Bassmann, 2005). However, based on our findings, we assume that a larger number of pesticides than expected persist for decades. It can be hypothesized that to a certain extent, non-extractable residues are formed by the parent chemical compound and, as part of a natural sorption–desorption continuum (Pignatello and Xing, 1996), are not totally excluded from environmental influences. In how far these residues in soils may pose an environmental threat by remobilization and leaching into adjacent ecosystems needs further detailed evaluation. We underline the need for further assessments of the long-term persistence of chemicals in the environment, their potential remobilization from a so-called “bound” state caused by e.g. changing climatic conditions, and their potential threat to non-target organisms in terms of risk assessment.

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